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We have used the equine infectious anemia virus (EIAV) system to antibody dependent enhancement (ADE) of lentivirus replication and disease by experimental EIAV vaccines, as an animal model for human AIDS vaccine studies. During the current project period we have performed in parallel experimental immunizations of Shetland ponies with several EIAV glycoprotein subunit vaccines to evaluate the risk of enhancement by the different subunit vaccines. The results of these studies demonstrate a high propensity for immune enhancement in ponies immunized with a baculovirus expressed recombinant gp90 vaccine or with a subunit vaccine containing HPLC-purified viral gp90. In contrast, a subunit vaccine based containing both of the EIAV envelope proteins (gp90 and gp45) and purified by lectin affinity chromatography produced a high degree of protection against virus challenge. Using immune serum from these immunized ponies, we have also developed *in vitro* ADE assays and evaluated the correlation of these assays with *in vivo* enhancement. Although the *in vitro* ADE assay remains to be optimized, initial results suggest that *in vitro* ADE assays may in fact distinguish between enhancing and protective immune responses in experimentally vaccinated ponies.

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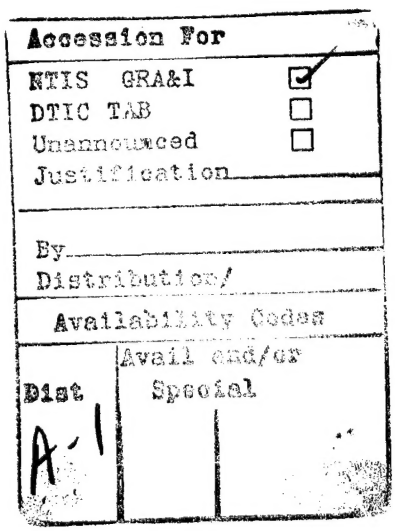

RC Montelaro 8/21/95

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A. INTRODUCTION

The potential for vaccine induced enhancement of infections by enveloped viruses has been documented in both human and veterinary medicine vaccine studies (Weiss et al. 1981, Halsted 1982, Porterfield 1986, Vennema et al. 1990). In the case of AIDS vaccine trials, there has been substantial concern about the possibility of immune enhancement of HIV-1 replication in target lymphocytes and macrophage cells by various mechanisms, including classic antibody dependent enhancement (ADE) or increases in the number or activation levels of target cell by vaccination (Bolognesi 1989, Burke 1992, Mascola et al 1993, Montelaro and Bolognesi 1995). Several studies have demonstrated that serum antibodies from HIV-1 infected patients and SIV infected monkeys can mediate ADE of virus replication in *in vitro* assays (Robinson et al. 1988, Homsy et al. 1988), Montefiori et al. 1990), and limited studies have suggested that the level of ADE may correlate with the progression of disease (Homsy et al 1990). However, the significance of the observed *in vitro* ADE to the levels of virus replication *in vivo* remains uncertain and controversial. Moreover, the lack of clear *in vitro* correlates for *in vivo* ADE poses a risk to proposed AIDS vaccine trials in that there are no predictive assays for immune responses that may result in either an increased susceptibility to infection by HIV-1 or acceleration of virus replication and disease upon infection by the virus. These questions are important to the U.S. Army in that HIV-1 infection constitutes a clear threat to military personnel, especially in developing countries where AIDS is epidemic. Thus the U.S. Army has established itself as a leader in the development of an effective AIDS vaccine.

During the past 10 years we have been using equine infectious anemia virus (EIAV), an animal lentivirus, as a model for the natural immunologic control of a lentivirus infection and as a system to evaluate various vaccine strategies against these viruses. In the course of evaluating various whole virus and subunit EIAV vaccines, we have observed a remarkable spectrum in vaccine efficacy ranging from sterile protection with attenuated and inactivated whole virus vaccines to severe enhancement of EIAV replication and disease by a baculovirus expressed surface glycoprotein vaccine (rgp90) (Issel et al. 1992, Wang et al. 1994). Although low levels of *in vivo* vaccine enhancement have been reported in CAEV (McGuire et al. 1986, McGuire 1987), visna virus (Narayan and Clements 1989), FIV (Hosie et al. 1992, Siebelink et al. 1995), and SIV (Schlienger et al. 1994, Gardner et al. 1995), the extent of enhancement in these animal lentiviruses is predominantly defined by slightly higher levels of virus replication with no clear correlation to exacerbation of disease. The EIAV system provides a uniquely relevant model in

which to examine the mechanisms of vaccine enhancement of lentivirus replication and disease and to evaluate vaccine strategies that minimize the potential for this enhancement phenomenon.

Thus, the original two-year specific aims of this specific research project are:

(i) To determine if prior immunization of ponies with the rgp90 vaccine enhances susceptibility to infection by homologous and heterologous strains of EIAV.

(ii) To determine if passive serum transfers from rgp90 immunized ponies to naive ponies can elicit enhancement of EIAV replication and/or disease by challenges with homologous and heterologous virus strains.

(iii) To examine the validity of *in vitro* ADE assays to predict *in vivo* enhancement and to use selected *in vitro* assays to elucidate the mechanisms and determinants of ADE in the EIAV system.

(iv) To evaluate in pony vaccine trials the protective and enhancing properties of selected subunit vaccines, including viral gp90/gp45 and baculovirus expressed recombinant glycoproteins containing all of gp90 and gp 45 (designated rgp135).

(v) To evaluate whether a more rigorous immunization regimen can eliminate the enhancement observed with the standard rgp90 immunization protocol.

B. PROGRESS REPORT

Towards these specific aims we have during the first year of this research project (July 15, 1994-July 14, 1995) addressed the following aspects of the experiments outlined in the original grant proposal:

Experiment #1. EIAV rgp90 immunizations of ponies to confirm and extend previous observations of enhancement of virus replication and disease and to produce immune serum for passive serum transfer experiments described in Experiment #3.

In this experiment we immunized 16 ponies at three week intervals with three 100 ug doses of the EIAV rgp90 vaccine in SAF-1 adjuvant, as described previously (Wang et al. 1993). Two weeks after the last immunization, large volumes of plasma were collected from four of the vaccinated ponies, two of which (no. 526 and 56) for use in passive transfer experiments (Experiment #3 below). At three weeks post immunization all 10 of the vaccinated ponies were inoculated with 300 animal i.d. of our standard virulent strain of EIAV, designated PV. Two naive ponies were also challenged with PV to serve as controls. Ponies were monitored daily over a two month observation period for clinical symptoms (fever, diarrhea, edema, etc.), and serum samples were taken each day for subsequent analyses of antibody responses and viremia levels. Figure 1 summarizes the clinical profiles of the ponies as monitored by rectal temperature (above 103° F considered as a fever) and platelet count (we have found thrombocytopenia to be an early and sensitive indicator of EIA in ponies).

In the case of the control ponies inoculated with the PV strain of EIAV, pony #87 displayed a characteristic clinical response with the appearance of fever and thrombocytopenia by about 35 days post challenge. This pony has subsequently displayed symptoms of chronic EIA and is being maintained to produce a long term infected pony for future studies of the evolution of protective immune responses in experimentally infected ponies. In contrast, pony #49 displayed an unexpected and unprecedented rapid and severe response to the standard EIAV challenge and had to be euthanized at 43 days post infection. In over 50 infections of ponies with the PV-EIAV at doses up to 10^5 pony infectious doses, we have never observed such a severe response in this standard experimental infection. Although we are pursuing an explanation as to the severity of disease in this control pony, it cannot be considered a typical response to EIAV infection and therefore should not at this time be used as a control infection to compare to the immunized ponies.

Thus, the initial clinical comparisons presented below are based on comparison to control pony #87.

According to the initial clinical data summarized in Figure 1, at least 4 of the rgp90 immunized ponies (no. 526, 66, 90, 65) appeared to have enhanced disease symptoms as evidenced by an accelerated and more severe decline in platelets and appearance of fever and other clinical symptoms compared to control pony #87. By three weeks post challenge, pony #526 was near death and had to be euthanized at 26 days post challenge. The severity of disease in ponies #66 and #90 also required that they be put down prior to the end of the usual 60 day observation period. Pony #65, while experiencing enhanced disease, was able to recover and survive for the full 60 day observation period. Four of the ten immunized ponies (no. 60, 120, 118, and 64) appeared to experience the usual course of clinical symptoms after the PV-EIAV challenge in that the appearance of their first febrile episode and thrombocytopenia paralleled that of the control pony #87. However, the severity of the initial clinical episodes in ponies #60 and #120 required that these animals be euthanized at 43 days post challenge, while ponies #118 and #64 recovered from their initial clinical episodes and were available for the full 60 day observation period. A final determination of any vaccine enhancement in this second group of 4 ponies must be based on the quantitation of viremia levels, assays that are now in progress. The final two (#73 and #56) of the ten immunized ponies failed to display either fever or a reduction in platelet counts during the two month observation period post challenge. Experiments to determine whether these ponies were protected from virus infection are in progress.

Assuming that the above clinical observations constitute a minimum estimation of vaccine enhancement, the results of this vaccine trial indicate that immunization with the EIAV rgp90 vaccine produced enhancement in at least 40% of the vaccinated ponies. It is probable that this percentage will increase as the viremia data become available. In any case, these vaccine trials confirm the propensity of the rgp90 vaccine to elicit immune responses that enhance rather than protect against EIAV exposure.

It should be noted here that these vaccine trials differ somewhat from previous studies in which 4 of 4 rgp90 immunized ponies displayed enhancement after challenge with the PV-EIAV (Wang et al 1994). Although the reason for this difference is uncertain at this time, we have begun an analyses of the virus specific antibody responses elicited in the two rgp90 vaccine trials.

Experiment #2. To determine if prior immunization with rgp90 increases susceptibility of ponies to infection by EIAV.

Experiments to address this issue are planned for year-2 of the research project.

Experiment #3. To determine if passive transfer of antibodies collected from ponies immunized with the rgp90 vaccine can mediate ADE in recipient ponies challenged with PV-EIAV.

Large volumes of plasma were collected from ponies #526 and #56 described in Experiment #1 above. These sera are now available for the passive transfer experiments, which should be initiated in the next couple of months. Interestingly, pony #526 apparently displayed clinical symptoms indicating severe enhancement, while pony #56 displayed no clinical symptoms over the 60 day observation period. Thus, these plasma serum samples can be used to determine if protection or enhancement can be passively transferred to naive ponies challenged with the PV-EIAV.

Experiment #4. Evaluation of *in vitro* assays of ADE in the EIAV system.

As part of our overall EIAV vaccine program we have been evaluating a number of *in vitro* antibody assays as correlates to protective or enhancing immune responses in experimentally infected or vaccinated ponies. These assays include determination of (i) antibody titer against viral envelope glycoproteins and vaccine immunogens, (ii) analyses of the antibody reactivity profiles with synthetic peptide segments the viral gp90, (iii) avidity measurements of antibodies specific for the viral envelope glycoproteins, and (iv) determination of the conformational dependence of antibodies reactive with the viral envelope glycoproteins. The results of these studies (Grund et al. 1995a,b) indicate a general pattern in which antibodies associated with protective immune responses react preferentially with native envelope glycoproteins, are directed predominantly to conformationally dependent determinants, and bind with a high avidity. In contrast, envelope-specific antibodies associated with enhancing immune responses react poorly with native viral envelope glycoproteins, are directed predominantly to linear determinants, and bind with low avidity. In addition, the gp-specific antibodies associated with protective and enhancing immune responses can be distinguished by their reactivity patterns with a panel of overlapping synthetic peptides encompassing the gp90 sequence of EIAV. These assays will be employed to characterize the antibody responses produced by the rgp90 vaccine in the current trials. It will be

especially interesting to determine if these *in vitro* assays can distinguish between immunized ponies that displayed various levels of vaccine enhancement. These assays are currently in progress.

While the above assays may provide relevant *in vitro* correlates of protection, the *in vitro* assay that should be the most relevant is to measure the ability of serum antibodies from vaccinated ponies to enhance EIAV replication in cultured equine macrophage. During the past year we have made a substantial effort to develop *in vitro* ADE assays that are sensitive and reproducible. In this quest, we have evaluated a number of different experimental parameters to optimize the assay. These studies are described below:

(i) Cell targets. EIAV is believed to infect only cells in the monocyte/macrophage lineage. In virus infected horses, the predominant sites of virus replication are tissues with abundant macrophage populations (eg., liver, kidney ,spleen, lung), while there is only very limited infection of blood monocytes, despite the relatively high viremia levels evident during clinical episodes (Montelaro et al 1993). As with other lentiviruses, EIAV replication in infected blood monocytes depends on activation and differentiation to macrophage (Maury 1994). Based on these observations and the relative availability of sources of macrophage from horses, we have initially developed our *in vitro* ADE assays using equine blood monocytes that have been isolated by adherence to the wells of microtiter plates and allowed to differentiate with or without the addition of cytokines. Extensive examinations of macrophage cultures prepared in this way reveal a marked variation in the cell populations, suggesting a very uneven and unpredictable level of differentiation from monocytes to macrophage. We are continuing to evaluate various methods of producing more uniform and predictable macrophage cultures from blood monocytes. The results of some initial ADE assays in these blood monocyte cultures are summarized later in this section. However, in light of the difficulties experienced to date, we are actively evaluating other sources of equine macrophage, including peritoneal and alveolar macrophage that can be isolated by lavage techniques. We are actively pursuing the standardization of these equine macrophage cultures.

(ii) Treatment of virus with serum antibodies. We have evaluated several different conditions for incubating serum antibodies with infectious virus, including the effects of using different serum dilutions and whether or not the cells are cultured in the presence of the immune serum. The maximum levels of ADE appear to be observed when the standard virus inoculum (moi of 0.001) is first incubated with a 1:4 dilution of immune serum at 37° C for 1 hr then allowed to infect the macrophage cells for two hours, after which the cells are cultured in the continued presence of the immune serum at a 1: 45 dilution in media for 7 days after infection. The

observation of ADE at low serum dilutions is in distinct contrast to *in vitro* ADE assays with immune serum from SIV or HIV-1 infections where ADE could only be observed at high serum dilutions, presumably under conditions that dilute neutralizing antibodies contained in the immune serum (Homsy et al. 1988, Robinson et al. 1988, Montefiori et al. 1990). This difference may reflect the relatively low levels of neutralizing antibodies observed in pony serum from ponies vaccinated under the conditions described here (Issel et al. 1992, Wang et al. 1994). Indeed, we have found that the level of in vitro ADE is reduced at higher dilutions of immune serum with end point titers below 1/100, indicating a correlation between ADE activity and the levels of EIAV-specific antibody in the assay. Representative in vitro enhancement data demonstrating the effects of different immune serum treatments on the level of enhancement observed is presented in Figure 2.

(iii) Measurements of virus replication. We have evaluated several methods of measuring the levels of virus production in an effort to maximize the detection of any antibody enhancement of EIAV replication in the macrophage cells. Initially we assayed virus production by measuring RT levels in supernatants at various time points post infection. The results of these experiments demonstrated the ability to detect virus production at 5-7 days post infection and a maximum enhancement level of 2-4-fold. In an effort to measure any enhancement of earlier steps in virus replication, we also used PCR to measure provirus formation in the presence and absence of immune serum. The results of these studies indicated that the while maximum enhancement levels of 2-4-fold were observed using the RT assay as a measure of virus production; provirus assays by PCR failed to detect enhancement any earlier than the RT assays. Therefore, we are currently using cell supernatant RT assays to measure the levels of virus production in the presence and absence of immune serum. We are also evaluating an immunostaining technique to measure the numbers of infected cells directly as a possible assay for detecting ADE at earlier time points post infection.

(iv) Measurements of viral cytopathicity. Another factor that we have observed to affect the apparent level of ADE in our *in vitro* assays is the rate and extent of CPE produced by virus infection. The basic observation is that increases in the level of virus production are accompanied by accelerated CPE that results in a decrease in viable cells. Therefore, the apparent enhancement of virus replication can be moderated by an accelerated CPE. At this point we are monitoring CPE and RT levels in parallel on a daily basis as complementary assays for enhanced virus production (see Table 1).

While we are continuing to optimize the *in vitro* ADE assay, we have used it to examine immune serum samples taken from the initial group of 4 ponies immunized with the rgp90 vaccine (Wang et al. 1994) and the 10 ponies most recently immunized with the rgp90 vaccine in Experiment 1 described above and immune serum from ponies vaccinated with either the LLgp or vgp90 vaccines. The data in Table 1 demonstrates some correlation between *in vitro* and *in vivo* enhancement in that immune serum from immunized ponies displaying clinical enhancement had a higher propensity for ADE *in vitro*, while serum antibodies elicited by protective vaccines (LLgp) were not associated with ADE *in vitro*. At this stage of the project, however, the *in vitro* assays can at best be described as a very insensitive measure of *in vivo* enhancement.

Experiment #5. To determine if immunization of ponies with various selected EIAV glycoprotein immunogens and different immunization protocols results in protection or enhancement of EIAV infection and disease.

To address this part of the research goals, we have immunized two groups of ponies with EIAV viral gp90 (vgp90) purified from gradient purified virus by reverse phase HPLC (Ball et al. 1988) and with the viral glycoproteins (gp90 and gp45) purified by lectin affinity chromatography (designated LLgp) from gradient purified EIAV (Montelaro et al. 1983, Issel et al. 1992). These immunizations were carried out in parallel with the baculovirus rgp90 vaccine trial described above in Experiment #1 using an identical immunization regimen and challenge protocol. Thus, the purpose of these experiments was to determine the protective or enhancing properties of EIAV subunit vaccines composed of viral envelope glycoproteins containing native equine glycosylation and purified under "relatively nondenaturing" conditions.

vgp90 vaccine. Six ponies were immunized with 3 doses (10 ug) of HPLC-purified gp90 isolated from the PR strain of EIAV. All of the ponies developed good antibody titers to the viral envelope glycoproteins as measured in ELISA (Table 1). To determine the efficacy of the vaccination, three ponies each were challenged with either the Pr or PV strain of EIAV. Initial clinical observations (Fig. 1 and Table 1) reveal that all of the challenged ponies became infected, indicating that the vgp90 vaccine failed to provide sterile immunity. In addition, all 3 of the PV-EIAV challenged ponies appeared to experience exacerbated clinical symptoms. Pony #88 developed sever disease and had to be put down at 26 days post challenge. Ponies # 412 and #449 developed fever and thrombocytopenia relatively early compared to the control pony #87 challenged with an identical inoculum of PV-EIAV. In the vgp90-vaccinated ponies challenged with the Pr-EIAV strain, two (no. 448 and 516) of the three animals appeared to experience an early febrile episode, while the third pony (no. 115) remained afebrile for the 60 day observation

period. However, none of the Pr-challenged ponies experienced a reduction in platelets. Taken together, these initial observations suggest that the vgp90 vaccine may have enhanced replication of the avirulent Pr-EIAV strain to a level causing mild disease. While a more definitive determination of vaccine enhancement in these vgp90 vaccine recipients must await more quantitative assays, these initial observations do indicate the potential danger of monomeric SU proteins as a vaccine immunogen.

LLgp vaccine. We previously reported (Issel et al. 1992) that an EIAV vaccine based viral envelope glycoproteins purified by lentil lectin affinity chromatography appeared to proved sterile protection against homologous virus challenge with the Pr strain of EIAV at 10^5 i.d., but failed to prevent protection or against heterologous challenge with the PV strain of EIAV at 300 i.d. In the case of the PV challenge, the LLgp vaccine produced a mixed result with respect to protection against the development of disease with one vaccinated pony remaining without clinical symptoms, two ponies displaying exacerbated clinical symptoms and viremia, and one pony developing disease and viremia similar to the nonimmunized control ponies inoculated with PV-EIAV. In this current vaccine trial, we wanted to revisit this particular subunit vaccine to examine the ability of the LLgp vaccine derived from PV virus to protect against PV challenge. Therefore, 4 ponies were immunized three times with 100 ug doses of PV-derived LLgp90 and then challenged with 300 i.d. of PV-EIAV. Initial clinical responses are summarized in Fig. 1 and Table 1. No clinical signs were observed in 3 of the 4 vaccinated ponies in this group. One pony (#84) had a mild febrile response coincident with a drop in platelets. While these initial results indicate that the LLgp vaccine can provide a high degree of protection against PV challenge, a determination of viremia levels is in progress to determine if sterile protection was achieved.

C. CONCLUSIONS

Any conclusions at this point must be considered tentative until we complete the analyses of *in vivo* and *in vitro* enhancement. However, even these initial results indicate certain important aspects of subunit vaccines:

(i) There is indeed a high propensity of vaccine enhancement of EIAV replication and disease associated with the baculovirus expressed rgp90 vaccine. At a minimum, 40% of immunized ponies displayed severe enhancement of disease, and it is likely that virus burden measurements will increase the level of enhancement observed in the group of ten ponies. These results confirm and extend previous studies with the rgp90 vaccine (Issel et al 1990).

(ii) EIAV vaccines based on highly purified monomeric gp90 failed to elicit protective immune responses, but instead appear to result in vaccine enhancement as observed with the baculovirus rgp90 vaccine above. These results suggest that using equine glycosylated gp90 does not eliminate the propensity of a SU subunit vaccine to result in vaccine enhancement.

(iii) The subunit vaccine based on lectin purified envelope glycoproteins (LLgp90) was able to provide a high degree of protection from homologous (PV) virus challenge, in agreement with previous LLgp vaccine trials using the Pr strain of virus as source of immunogen and as challenge virus (Issel et al. 1992).

(iv) The results of the LLgp vaccine trial confirm the high efficacy of protection afforded by this subunit vaccine against homologous virus challenge, i.e., PV LLgp and PV challenge virus. In our previous study with a Pr-LLgp vaccine, we noticed sterile protection against homologous (Pr) virus challenge. In contrast, the Pr-LLgp vaccine failed to provide protection from infection and disease in immunized ponies challenged with the heterologous PV-EIAV strain. Whether the differences in the observed efficacy of protection was due to the antigenic or virulence differences between the Pr and PV strains of EIAV were uncertain. However, the current observation of protection (at least from disease) by a PV-LLgp vaccine against PV challenge is consistent with the concept that antigenic differences may in fact be the major factor in determining whether or not a vaccine is effective. We will be able to evaluate this model more thoroughly once we determine whether or not the PV-challenged ponies in this trial were also protected from infection.

(v) Initial results from *in vitro* ADE assays indicate a discrimination between protective (LLgp vaccine) and enhancing (vgp90 and rgp90 vaccines) antibody responses, as measured by levels of virus production and by the extent of CPE in cultured macrophage. However, the reproducibility and sensitivity of these *in vitro* assays require further improvements.

(vi) Based on these very initial observations, one might speculate that vaccine protection in the EIAV system can only be achieved when both viral gp90 and gp45 are present in the context of a multimeric complex, eg, inactivated whole virus or the LLgp preparations. In contrast, monomeric gp90, either viral or baculovirus recombinant, elicits immune responses that result in enhancement rather than protection.

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E. PUBLICATIONS AND METING ABSTRACTS RELATED TO THIS GRANT.

Montelaro, R., Grund, C., Raabe, M., Woodson, B., Cook, R., Cook, S., Issel, C. (1995) Characterization of protective and enhancing immune responses to EIAV resulting from experimental vaccines (A brief review). AIDS Res. Human Retroviruses, in press.

Grund, C., Lechman, E., Pezzuolo, N., Issel, C. and Montelaro, R. (1995) Fine specificity of EIAV gp90-specific antibodies associated with protective and enhancing immune responses in experimentally infected and immunized ponies. J.Gen. Virol., in press.

Montelaro, R. (1995) Protective and enhancing immune responses to EIAV in experimentally vaccinated ponies. International Meeting on Comparative Retroviral Vaccines, Fondation Merieux, Annecy, France. (Meeting Abstract).

F. PERSONNEL PAID BY THIS GRANT.

University of Pittsburgh:
Ronald C. Montelaro, Ph.D., 10%, Principal Investigator
Bruce Woodson, Ph.D., 50%, Research Associate
Michelle Raabe, 50%, Graduate Research Assistant

University of Kentucky:
R. Frank Cook, Ph.D., 10%, Research Specialist
Sheila J. Cook, M.S., 100%, Research Technician/Lab. Manager
Gary Thomas, 50%, Animal Caretaker
Brian Meade, 50%, Animal Caretaker

Note: No graduate degrees have resulted from this grant support during this reporting period.

G. APPENDIX

1 Table

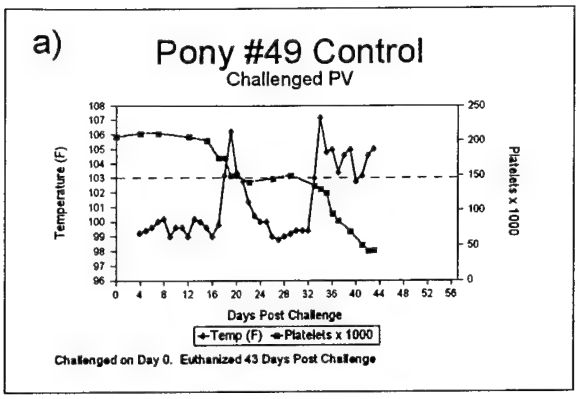
3 Figures

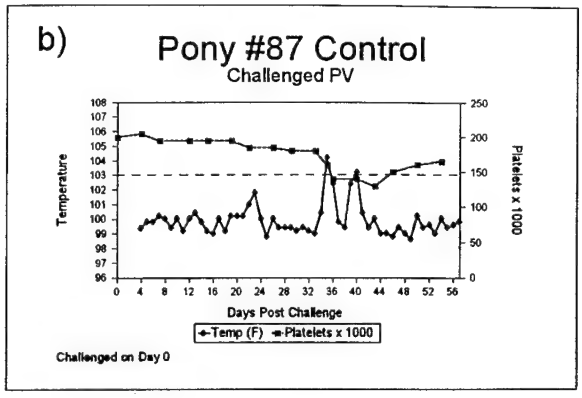
Table 1.
INITIAL OBSERVATIONS OF EIAV SUBUNIT VACCINE TRIALS

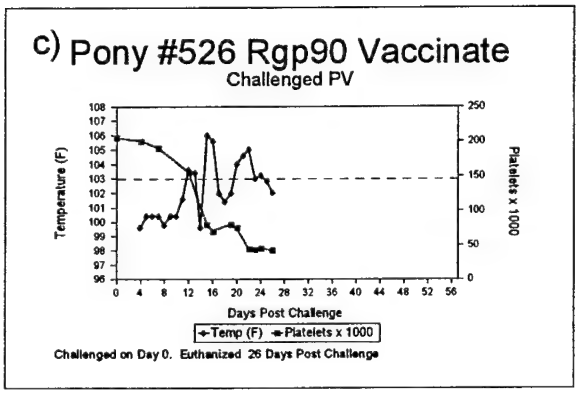
Vaccine Group	Pony No.	Challenge Virus[a]	Enhancement of Clinical Symptoms[b]	Antibody Titers[d] rgp90	vgp	In Vitro ADE[e]	InVitro CPE[f]
1991 rgp90	1-5	PV	Severe	1/3200	1/1600	+	+
	1-6	PV	Severe	1/6400	1/6400	+/-	=
	1-7	PV	Severe	1/1600	1/50	-	=
	21	PV	Severe	1/3200	1/50	+/-	=
1995 rgp90	526	PV	Severe	1/12800	1/12800	-	+/-
	66	PV	Severe	1/12800	1/51200	+	+
	90	PV	Severe	1/25600	1/6400	+/-	=
	60	PV	Severe	1/12800	1/6400	+	=
	65	PV	Severe	1/12800	1/25600	+/-	+
	120	PV	Mild	1/6400	1/6400	-	+
	118	PV	Mild	1/25600	1/3200	+	+
	64	PV	Mild	1/12800	1/6400	-	+/-
	73	PV	Protective	1/3200	1/800	-	+/-
	56	PV	Protective	1/12800	1/12800	+	=
1995 llgp90	45	PV	Protective	ND[c]	>1/51200	-	-
	84	PV	Mild	ND	>1/51200	-	-
	95	PV	Protective	ND	1/51200	-	-
	100	PV	Protective	ND	1/51200	-	-
1995 vgp90	88	PV	Severe	ND	1/1600	-	-
	449	PV	Mild	ND	1/6400	-	-
	412	PV	Mild	ND	1/3200	-	-
	448	PR	Mild	ND	1/6400	-	-
	115	PR	Mild	ND	1/800	-	-
	516	PR	Mild	ND	1/3200	+/-	-

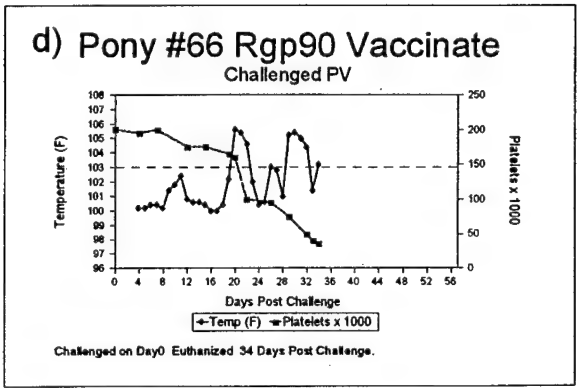
[a] I.V. challenge with either 300 i.d. of the EIAV strain PV or 10^5 i.d. of PR-EIAV.
[b] Evaluation of clinical responses in immunized ponies compared to nonimmunized controls with respect to appearance and severity of clinical symptoms (fever, diarrhea, etc.) and thrombocytopenia. Severe enhancement describes acceleration of fever and thrombocytopenia; mild enhancement describes acceleration of either the development of fever or thrombocytopenia; none would describe fever and thrombocytopenia responses similar to non-immunized ponies infected with virus (we did not observe this type of response) and protected describes the absence of detectable fever or thrombocytopenia.
[c] ND - Not done as yet.
[d] Endpoint titrations of immune serum reactivity with either baculovirus expressed rgp90 or EIAV envelope glycoproteins (vgp) in Con A ELISA.
[e] Analyses of in vitro ADE activity in serum samples taken from immunized ponies as averaged over five different assays. (+) indicates enhancement greater than two fold, (+/-) indicates enhancement less than two fold and (-) indicates that no enhancement was observed.
[f] Analyses of CPE in the in vitro ADE cell wells. This data is from a single experiment and should not be directly compared to the in vitro ADE data. (+) indicates advanced cytotoxicity when compared to control, (+/-) indicates some cytoxicity, (=) indicates no cytotoxicity and a (-) indicates dramatically less cytoxicity than that observed in the control.

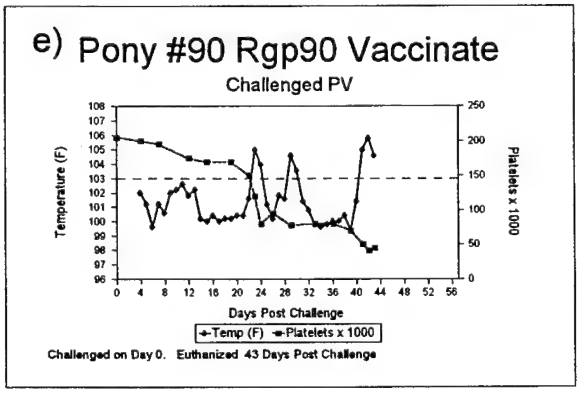
Figure 1. Temperature and platelet data of ponies after challenge with EIAV.











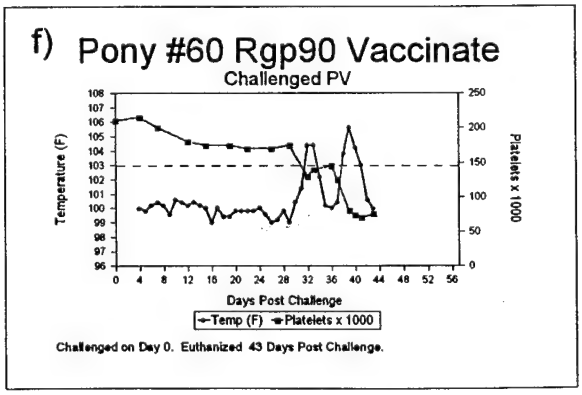
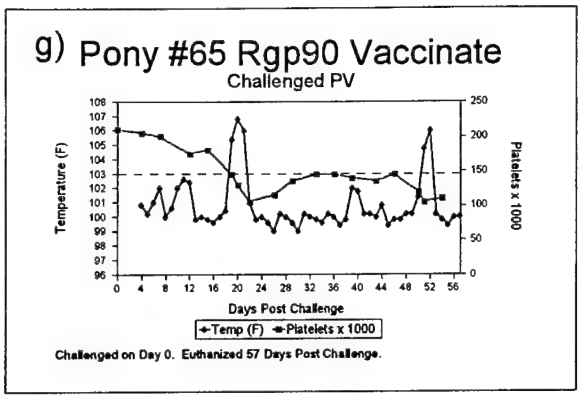
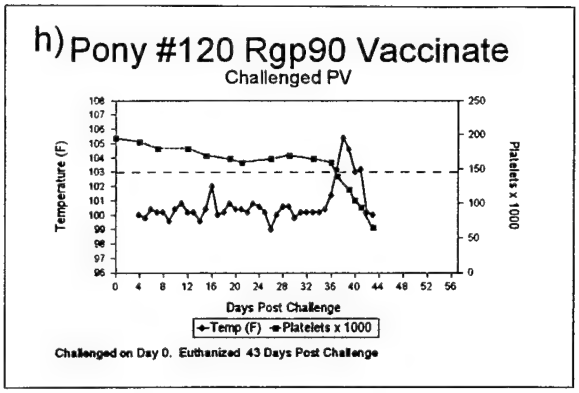
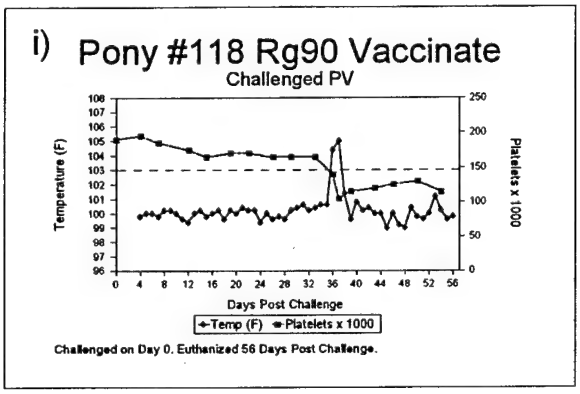
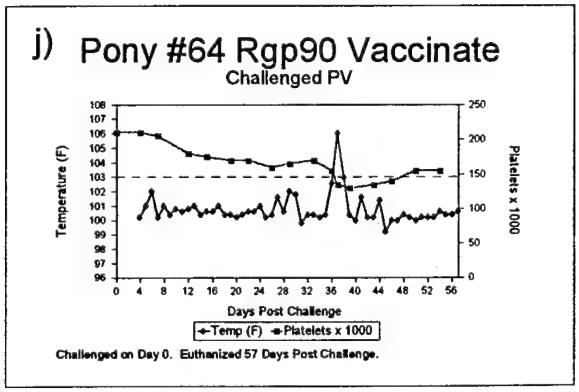


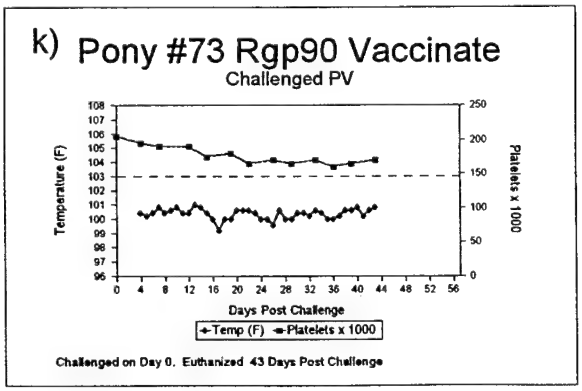
Figure 1. Temperature and platelet data of ponies after challenge with EIAV cont.











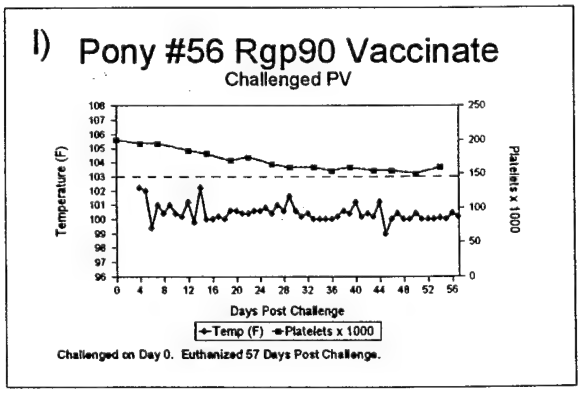
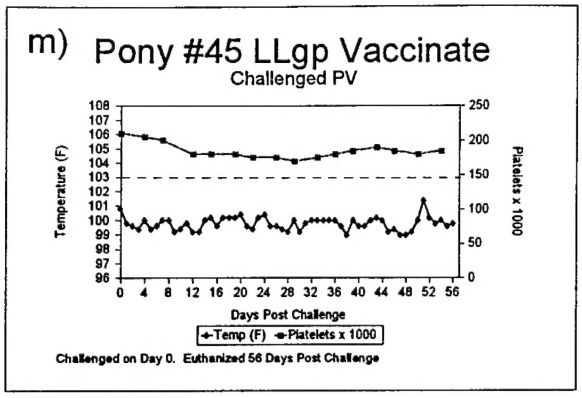
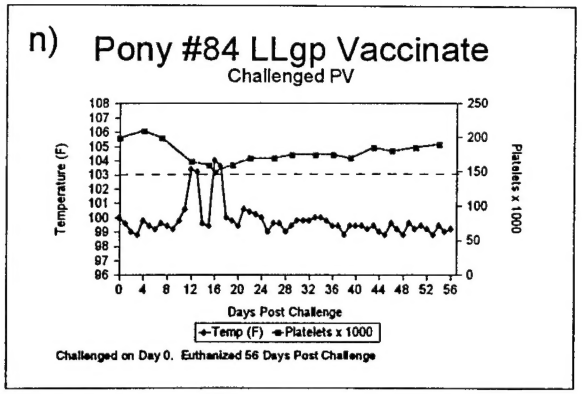
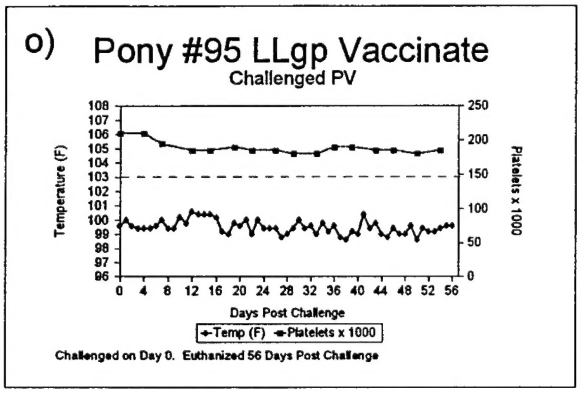
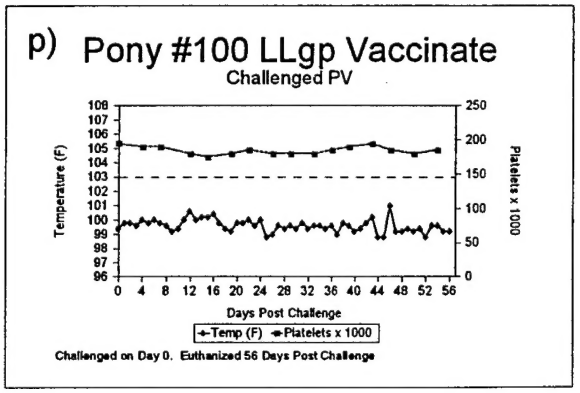


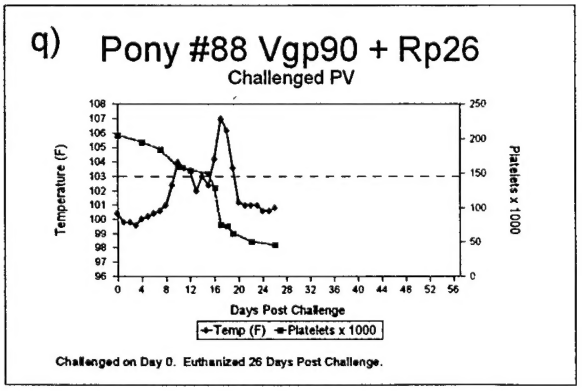
Figure 1. Temperature and platelet data of ponies after challenge with EIAV cont.











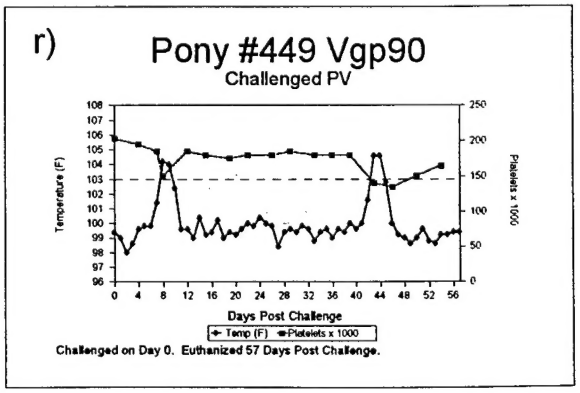
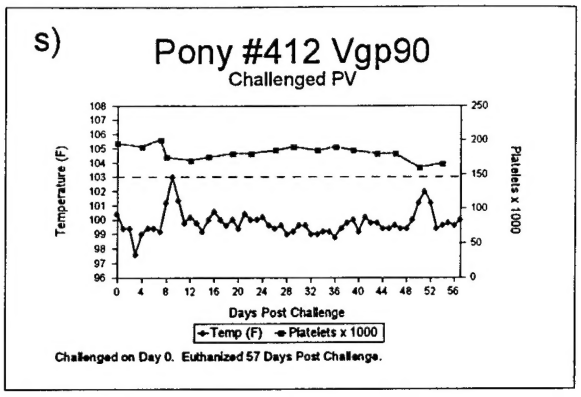
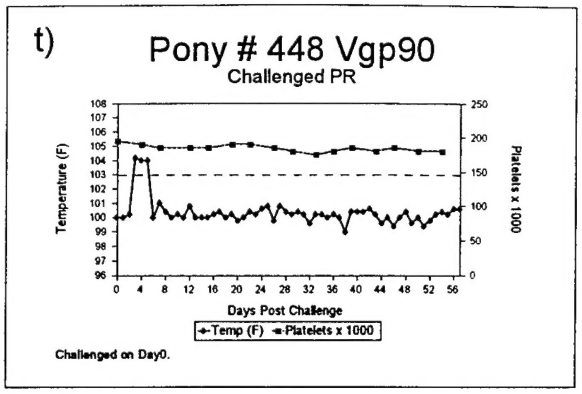
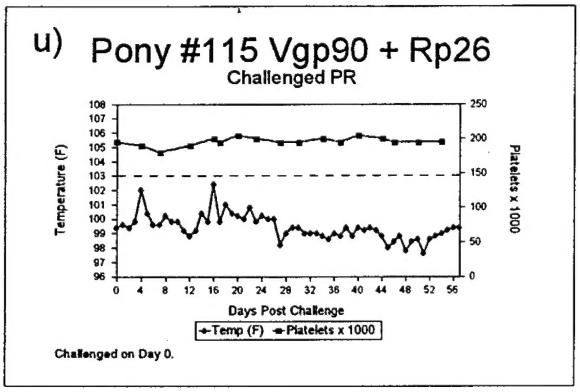
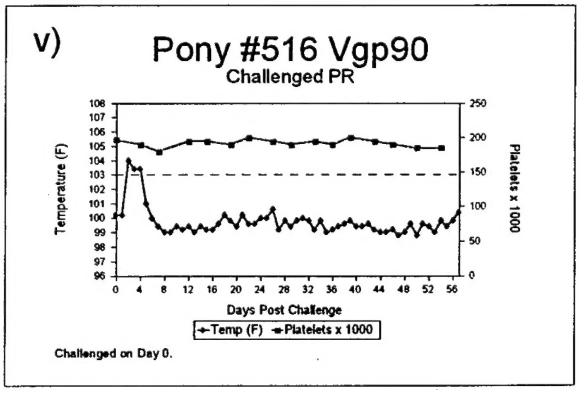


Figure 1. Temperature and platelet data of ponies after challenge with EIAV cont.









Analysis of Serum Dilutions in In Vitro ADE Assay

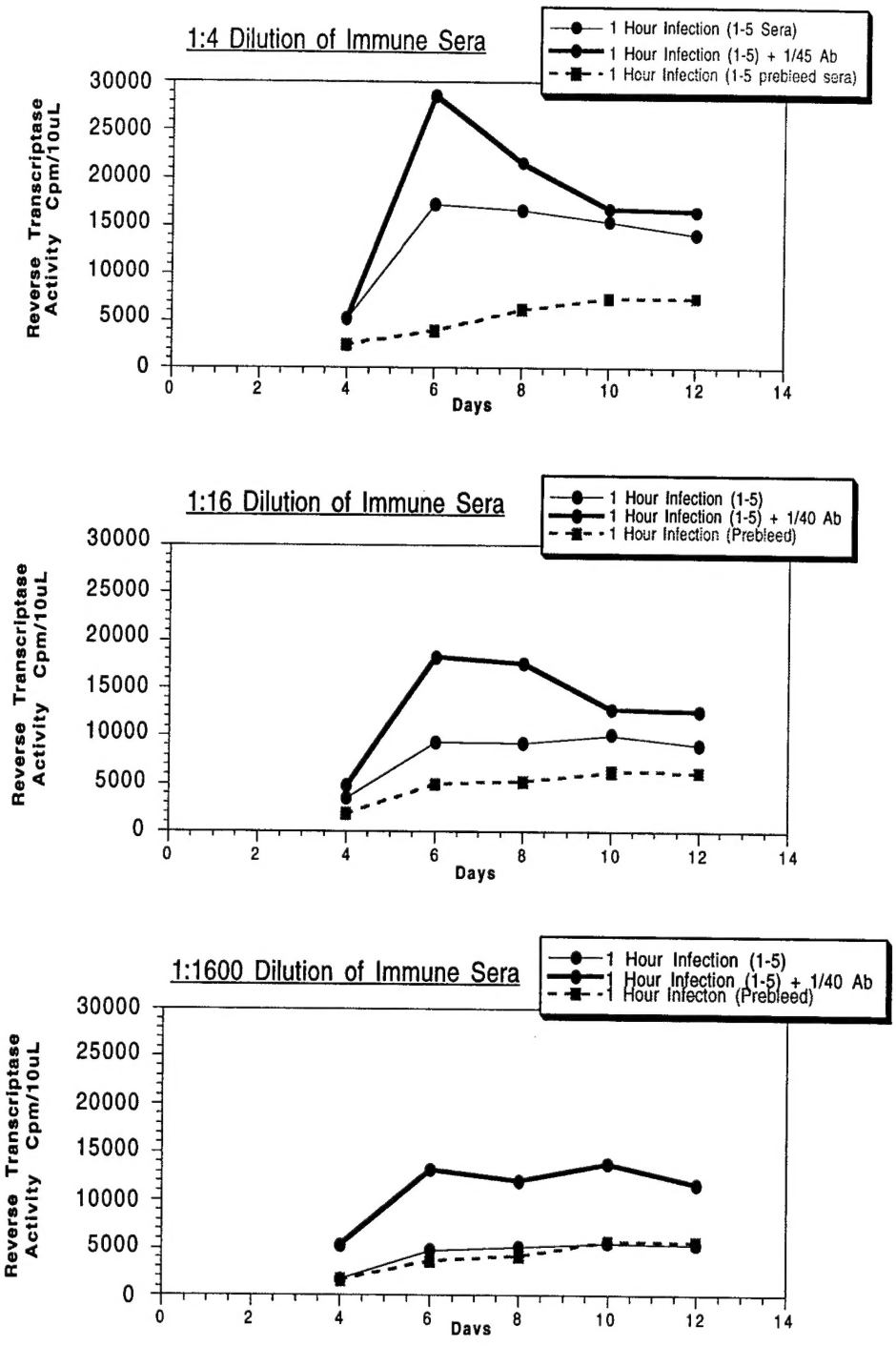
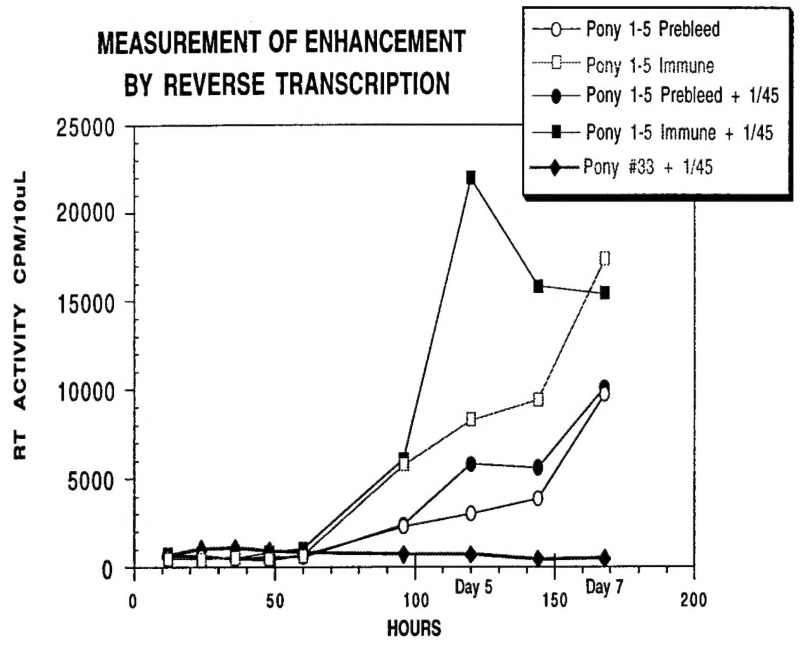


Figure 2. Monocyte/macrophage cultures were infected with mixtures of PV virus (Moi 0.001) and two fold dilutions of rgp90 immune serum or prebleed serum and then cultured in the presence or absence of a 1/45 dilution of the immune sera. The cultures were incubated for a total of 12 days during which reverse transcriptase activity was assayed in the supernatents at various time points.

The lower dilutions of immune sera resulted in greater enhancement of the EIAV infections indicating a low concentration of neutralizing antibodies in these immune sera. When the cells were continuously cultured in the presence of the enhancing antibodies the degree of enhancement was increased by a factor of two suggesting the enhancement process to be an exponential one that continually multiply over time. As seen previously, enhancement in the ADE cultures titered out at serum dilutions above 1/100 but again, the continued presence of antibody in the culture media maintained the enhancement effect up to a dilution of 1/1600.



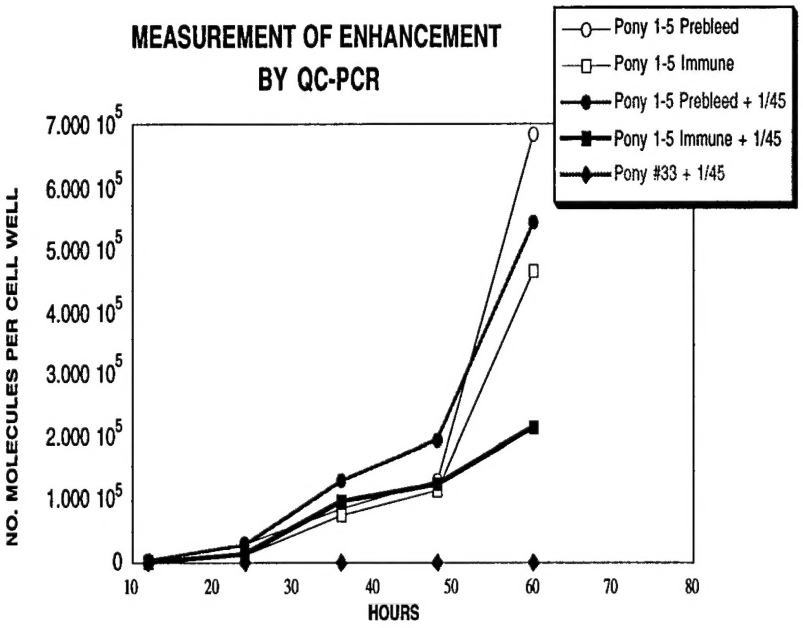


Figure 3. Monocyte/macrophage cultures were infected with a mixture of PV virus (Moi 0.001) and a 1/4 dilution of Pony 1-5 immune serum (1991 rgp90 trial) or 1-5 prebleed serum. Another set of cell wells were infected with a mixture of virus and Pony #33 serum which is known to contain neutralizing antibodies. The cells were then cultured in the presence or absence of a 1/45 dilution of the appropriate serum. At 12 hour intervals up to 60 hours, the cell supernatents were harvested and assayed for reverse transcriptase activity and the cells were lysed and examined by PCR to determine levels of viral DNA. One set of cell wells was retained for a total of 7 days and the RT activity in the well was assayed at various time points.

The resultant RT data shows that enhancement was achieved in this experiment to a maximum level of four fold in the continued presence of antibody, as seen previously. No RT activity was ever detected in the well infected in the presence of Pony #33 sera, thus indicating that neutralizing antibodies were present and, more importantly, that these antibodies do not result in nonspecific enhancement.

The PCR data obtained shows no significant difference in viral DNA levels between the different cell populations up to 60 hours post infection. Samples post 60 hours were not evaluated here. Therefore, PCR was unable to detect enhancement in this experiment before it could be detected by measuring RT activity in the cell supernatents.